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FINAL REPORT

Grant #: N00014-00-1-0314

PRINCIPAL INVESTIGATOR: Tania Zenteno-Savín, Ph.D.

GRANT TITLE: Coping with repeated ischemia and reperfusion: Physiology of Free Radicals in Diving Birds and Mammals

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OBJECTIVE: The specific mechanisms involved in protecting marine bird and mammal tissues from generation of free radicals and their deleterious effects are not known and are the subject of this research. The short-term objectives of this study are to analyze the rate of production of superoxide radical as an index of synthesis of free radicals, in tissues from marine birds and mammals; to examine the degree of lipid peroxidation as an index of free radical-induced damage, in tissues from marine birds and mammals; and to do a selective analysis of the antioxidant defenses which may endow marine birds and mammals with the tolerance to repeated cycles of ischemia/reperfusion associated to diving.

APPROACH: Using colorimetric techniques, we are evaluating the rate of production of superoxide radicals, lipid peroxidation, and the activities of the main antioxidant enzymes in extracts of tissues from ringed seals, *Phoca hispida*, and emperor penguins *Aptenodytes forsteri*. These determinations are made under basal conditions and under simulated oxidative stress.

ACCOMPLISHMENTS: Baseline production of superoxide radical ($O_2^{\cdot-}$) and lipid peroxidation (TBARS) were obtained from 8 emperor penguins, 3 northern elephant seals and 3 California sea lions; data is available for swimming (S) and non-swimming (NS) muscle biopsies from these species. These results were obtained in collaboration with Dr. Paul Ponganis at the University of California San Diego-Scripps Institution of Oceanography. We have analyzed the production of $O_2^{\cdot-}$, TBARS and total antioxidant capacity (AOX) in samples of heart, kidney and muscle from ringed seals, that have been obtained with the cooperation of alaskan subsitence hunters. Samples from 17 seals were obtained and analyzed, and compared with results obtained from 13 pigs as a model of terrestrial mammal. Oxidative stress was simulated in these samples by incubating with xanthine/xanthine oxidase (X/XO). Activity of the antioxidant enzimes catalase, superoxide

dismutase and glutathione S-transferase were analyzed in tissues samples from ringed seals. These results are product of collabration with Dr Robert Elsner, University of Alaska Fairbanks. Additionally, in collaboration with Dr. Peter Johnson, Ohio University, we are studying the presence of hipoxia-inducible factor (HIF) in ringed seal tissues.

$O_2^{\cdot-}$ production in ringed seal and elephant seal was higher in S than NS muscle ($P<0.1$). $O_2^{\cdot-}$ production in California sea lion and penguin samples was higher in NS than in S muscle ($P<0.1$). $O_2^{\cdot-}$ production was higher in all marine species than in domestic pig ($P<0.1$). TBARS were higher in NS than S muscle in California sea lion ($P<0.1$). Emperor penguin S muscle had higher TBARS than NS muscle ($P<0.1$). While seal, sea lion and penguin muscle showed much higher $O_2^{\cdot-}$ production than domestic pig muscle, tissue damage, as evidenced by TBARS levels, was not proportionally increased in the marine bird and mammals. Antioxidant capacity was analyzed in ringed seal and domestic pig muscle samples, and was found to be higher in ringed seal ($P<0.1$).

<u>CONCLUSIONS</u>: In reponse to cyclic episodes of ischemia/reperfusion associated with their frequent dives, one might expect that seal, sea lion and penguin tissues have biochemical and/or physiological adaptations for tolerance of, or protection from, the potential oxidative insult. In this study we found higher production of $O_2^{\cdot-}$ in muscle from ringed seal, elephant seal, California sea lion and emperor penguin than in muscle from the domestic pig. Despite the higher $O_2^{\cdot-}$ production found in penguin, seals and sea lion, tissue damage was not proportionally increased in the marine bird and mammals, as evidenced by TBARS levels. The differences in AOX between pig and ringed seal muscle, provide a suitable explanation for this protection against $O_2^{\cdot-}$. Differences between S and NS may be due to fiber type distribution. Alternatively, differences among species may be related to diving capacities. Overall, the results from this study suggest that tolerance of dive-associated ischemia/reperfusion in marine organisms may depend on enhanced intermediate scavenging of reactive oxygen species.

Oxidative stress was simulated in these samples by incubating with xanthine/xanthine oxidase (X/XO). Treatment with X/XO had no effect on TBARS in ringed seal kidney or heart. These results show that ringed seal muscle, heart and kidney can be induced *in vitro* to generate reactive oxygen species, and suggest that the living seal's protective defenses may depend on $O_2^{\cdot-}$ production, similar to the protective effect of experimental preconditioning, or on enhanced intermediate scavenging, as evidenced by the larger AOX found in ringed seal tissues compared to pig tissues. Additionally, activity of the antioxidant enzimes catalase,

superoxide dismutase and glutathione S-transferase were analyzed in tissues samples from ringed seals. These results are in accordance to those obtained by other researchers (e.g., Wilhelm-Filho et al., 2002), and suggest that marine mammals have an enhanced antioxidant capacity that allows them tolerance of the potential oxidative stress associated to repeated cycles of ischemia-reperfusion.

SIGNIFICANCE: This project seeks to understand the metabolic adaptations and physiologic mechanisms that allow marine birds and mammals to cope with the hypoxia, ischemia/reperfusion and oxidative stress associated to breath-hold diving, which would help us understand how this factors affect human divers and how to deal with specific pathologies associated with exposure to hypoxia, ischemia/reperfusion and oxidative stress. For example, oxygen toxicity, an important problem for human divers, is at the cellular level an effect of the free radicals; therefore, marine birds and mammals can be thought of as models for the study of such condition.

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